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Association Between Polymorphisms of Ethanol-Metabolizing Enzymes and Susceptibility to Alcoholic Cirrhosis in a Korean Male Population

Alcohol is oxidized to acetaldehyde by alcohol dehydrogenase (ADH) and cytochrome P-4502E1 (CYP2E1), and then to acetate by aldehyde dehydrogenase (ALDH). Polymorphisms of these ethanol-metabolizing enzymes may be associated with inter-individual difference in alcohol metabolism and susceptibility to alcoholic liver disease. We determined genotype and allele frequencies of *ALDH2, CYP2E1*, *ADH2*, and *ADH3* in male Korean patients with alcoholic cirrhosis (n=56), alcoholics without evidence of liver disease (n=52), and nondrinkers (n=64) by using PCR or PCR-directed mutagenesis followed by restriction enzyme digestion. The prevalences of heterozygous ALDH2*1/*2 plus homozygous ALDH2*2/*2 in patients with alcoholic cirrhosis (7.1%) and alcoholics without evidence of liver disease (3.8%) were significantly lower than that in nondrinkers (45.3%). The c2 allele frequencies of the CYP2E1 in alcoholic cirrhosis, alcoholics without evidence of liver disease, and nondrinkers were 0.21, 0.20, and 0.20, respectively. Allele frequencies of ADH2*2 in the three groups were 0.78, 0.74, and 0.77 and those of ADH3*1 were 0.94, 0.98, and 0.95. Therefore, we confirmed the observation that the *ALDH2*2* gene protects against the development of alcoholism. However, the development of cirrhosis in Korean alcoholic patients was not associated with polymorphisms of ethanol-metabolizing enzymes.

Key Words : *Alcohol Dehydrogenase; Aldehyde Dehydrogenase; Cytochrome P-450 CYP2E1; Liver Cirrhosis, Alcoholic; Polymorphism (Genetics)*

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INTRODUCTION

Alcoholism is an important cause of chronic liver disease, but only 10% to 20% of alcoholics develop cirrhosis (1). Alcohol is oxidized initially to acetaldehyde, principally by alcohol dehydrogenase (ADH) and cytochrome P-4502E1 (CYP2E1), and then to acetate by aldehyde dehydrogenase (ALDH). Acetaldehyde, a highly toxic metabolite of ethanol, has been implicated in the pathogenesis of alcoholic liver disease (2). Theoretically, acetaldehyde accumulation in the liver after chronic alcohol ingestion is determined by the rates of its formation and removal, catalyzed by ADH and CYP2E1, and ALDH, respectively. A twin study suggested a genetic basis for individual differences in susceptibility to alcoholic cirrhosis (3), and genetic polymorphisms of enzymes involved in the oxidative metabolism of ethanol to acetate are known (4-7). Therefore, these genetically determined variations in an individual's ability to metabolize alcohol might influence the prevalence of alcoholic liver disease in alcoholics.

Persons with deficient mitochondrial ALDH (ALDH2*2) are protected against the development of alcoholism, because of the unpleasant symptoms (facial flushing, tachycardia, nausea, etc.) after drinking (8, 9). However, it was also suggested that habitual drinkers with ALDH2 deficiency might be at higher risk of alcohol-associated liver disease. The *Rsa*I and *Pst*I polymorphisms in the 5′-flanking region of *CYP2E1* are related to the transcriptional regulation of the enzyme, and the transcriptional activity of the *c2* gene is 10 times higher than that of the *c1* gene in vitro (7). Also for ADH, the ADH2*2 and ADH3*1 alleles encode the more active β_2- and γ_1-ADH subunits, respectively, and these predominate in Asian populations (10-12). Therefore, these variants of the CYP2E1 and ADH isozymes metabolize ethanol to acetaldehyde more rapidly and may result in an increased accumulation of acetaldehyde in the liver after drinking. Therefore, individuals with these isozymes may be either more susceptible to alcoholic cirrhosis or protected against the development of alcoholism. However, the roles of the genetic polymorphisms of these enzymes, CYP2E1 and ADH, in the development of alcoholism and alcoholic liver disease are also controversial (9, 13-17).

Ethanol metabolism is a dynamic process involving several enzymes, therefore, to determine the net amount of aldehyde

produced, it is necessary to simultaneously analyze the polymorphisms of these ethanol-metabolizing enzymes. Therefore, we analyzed the genetic polymorphisms of all these enzymes simultaneously to assess whether they affect predisposition to alcoholism and/or alcoholic cirrhosis susceptibility.

MATERIALS AND METHODS

Subjects

Blood samples were obtained from 56 patients with alcoholic cirrhosis, 52 alcoholics without evidence of liver disease, and 64 nondrinkers. Because there is evidence that women have increased susceptibility to alcoholic cirrhosis (18), females were excluded from the study. Patients with alcoholic cirrhosis (alcoholic cirrhosis group) enrolled into this study consumed more than 80 g of ethanol per day for more than 10 yr and had clinical evidences of portal hypertension such as esophageal varices, ascites, or hepatic encephalopathy. All the alcoholic cirrhotic patients were negative for the hepatitis B surface antigen and the antibody to hepatitis C virus by the radioimmunoassay (Abbott Laboratories, North Chicago, IL) and the second-generation enzyme immunoassay (Abbott Laboratories), respectively. Alcoholic patients without clinical evidence of liver disease (alcoholics group) also consumed more than 80 g of ethanol per day for more than 10 yr, and had normal liver function test values without clinical or radiological (ultrasonography or CT) evidence of liver disease. Age-matched healthy males who drank less than 80 g per week were arbitrarily defined as nondrinkers.

There was no significant difference in age, total and mean daily amount of ethanol intake, and duration of drinking between the alcoholic cirrhosis group and the alcoholics group (Table 1).

Determination of *ALDH2*, *CYP2E1*, *ADH2*, and *ADH3* genotypes

The buffy coat was separated from 10 mL of anti-coagulated blood containing acid citrated dextrose solution, and the DNA of white blood cells was extracted by the method of Blin et al. (19).

ALDH2 genotypes were determined according to Chao et al. (9) using a PCR-directed mutagenesis method with some modifications. Primers YC1 (5'-CCACACTCACAGTTTTGAATT-3') and YC2 (5'-GGCTACAAGAATTCGGGGAGT-3') were used to amplify the *ALDH2* gene. After amplification, the genotypes were determined by *Eco*RI restriction fragment length polymorphisms.

The *Rsa*I and *Pst*I polymorphisms of the *CYP2E1* gene were determined according to Hayashi et al. (7), using primers J8 (5'-TTCATTCTGTCTTCTAACTGG-3') and J9 (5'-CCA GTCGAGTCTACATTGTCA-3'). The amplified products were digested with *Rsa*I or *Pst*I.

ADH2 and *ADH3* genotypes were determined according to Groppi et al. (20) with some modifications. For detection of the ADH2*2 allele, *ADH2* exon 3 was amplified with primers 303 (5'-ATTCTGTAGATGGTGGCTGT-3') and 247 (5'-GAAGGGGGGTCACCAGGTTG-3'), and the products were digested with *Mae*III. For identification of the ADH2*3 allele, *ADH2* exon 9 was amplified with primers HE39 (5'-TGGACTCTCACAACAAGCATGT-3') (21) and 290 (5'-TTTCTTTGGAAAGCCCCCAT-3'), and then PCR-directed mutagenesis using primers 352 (5'-TCTTTCCTAT TGCAGTAGC-3') and 290 was performed with 1/500 of the first amplification mixture as a template. The second amplification products were digested with *Alu*I. For determination of the *ADH3* genotypes, primers 321 (5'-GCTTTAAGAG-TAAATATTCTGTC-CCC-3') and 351 (5'-AATCTACCTC-TTTCCGAAGC-3') were used to amplify the *ADH3* loci, and then the amplified products were digested with *Ssp*I.

Table 1. Comparison of age and alcohol consumption

Group	No. of cases	Age (yr)	Alcohol consumption		
			Total (kg)	Daily (g)	Duration (yr)
Alcoholic cirrhosis	56	52.2±9.3	1357.4±1196.5	165.6±91.8	22.7±9.9
Alcoholics	52	51.7±10.1	1215.0±807.1	152.8±99.6	22.8±9.8
Nondrinkers	64	50.4±7.5	31.8±26.5	4.1±2.8	18.8±12.5

Results are expressed as means±SD, No., Number

Table 2. Genotype and allele frequencies of *ALDH2*

Group	Genotype			Allele frequency	
	ALDH2*1/*1	ALDH2*1/*2	ALDH2*2/*2	ALDH2*1	ALDH2*2
Alcoholic cirrhosis (n=56)	52 (92.9%)	4 (7.1%)	0 (0%)	0.96	0.04*
Alcoholics (n=52)	50 (96.2%)	2 (3.8%)	0 (0%)	0.98	0.02*
Nondrinkers (n=64)	35 (54.7%)	25 (39.1%)	4 (6.2%)	0.74	0.26

*p=0.001 compared with nondrinkers. ALDH2, aldehyde dehydrogenase 2

Statistical analysis

For group comparison, χ^2 test or Student's t-test was used. A p value of <.05 was considered statistically significant.

RESULTS

Genetic polymorphism of *ALDH2*

Table 2 shows the genotype and allele frequencies of *ALDH2* in each group. Whereas the genotype frequency of heterozygous ALDH2*1/*2 plus homozygous ALDH 2*2/*2 was 45.3% in nondrinkers, they were 3.8% and 7.1% in the alcoholics group and the alcoholic cirrhosis group, respectively (p=0.001). The allele frequencies of ALDH2*2 were 0.04 in the alcoholic cirrhosis group, 0.02 in the alcoholics group, and 0.26 in nondrinkers.

Genetic polymorphism of *CYP2E1* with *Rsa*I/*Pst*I

There was no difference in genotype distribution and allele frequencies among the three groups. The allele frequencies of *c2* gene were 0.21 in the alcoholic cirrhosis group, 0.20 in the alcoholics group, and 0.20 in nondrinkers (Table 3).

Genetic polymorphism of *ADH2*

The allele frequencies of ADH2*2 were 0.78 in the alcoholic cirrhosis group, 0.74 in the alcoholics group, and 0.77 in nondrinkers. There was no significant difference in genotype distribution and allele frequencies of *ADH2*2* gene among the three groups (Table 4). Neither homozygote nor heterozygote for ADH2*3 allele was found in the Korean population.

Genetic polymorphism of *ADH3*

The allele frequencies of ADH3*2 were 0.06 in the alcoholic cirrhosis group, 0.02 in the alcoholics group, and 0.05 in nondrinkers. There was no significant difference in genotype distribution and allele frequencies of *ADH3*2* gene among the three groups (Table 5).

Genotype and allele frequencies of *CYP2E1* in subjects with ADH2*2/*2, ADH3*1/*1, and ALDH2*1/*1

We analyzed the genotype and allele frequencies of *CYP2E1* in subjects with ADH2*2/*2, ADH3*1/*1, and ALDH2*1/*1 to exclude the possible influence of ethanol-metabolizing enzymes other than CYP2E1. There was no significant difference among the three groups (Table 6).

Table 3. Genotype and allele frequencies of *CYP2E1*

Group	Genotype			Allele frequency	
	A (c1/c1)	B (c1/c2)	C (c2/c2)	c1	c2
Alcoholic cirrhosis (n=56)	34 (60.7%)	21 (37.5%)	1 (1.8%)	0.79	0.21
Alcoholics (n=52)	32 (61.5%)	19 (36.6%)	1 (1.9%)	0.80	0.20
Nondrinkers (n=64)	41 (64.0%)	22 (34.4%)	1 (1.6%)	0.80	0.20

CYP2E1, cytochrome P-4502E1

Table 4. Genotype and allele frequencies of *ADH2*

Group	Genotype			Allele frequency	
	ALDH2*1/*1	ALDH2*1/*2	ALDH2*2/*2	ALDH2*1	ALDH2*2
Alcoholic cirrhosis (n=56)	7 (12.5%)	11 (19.6%)	38 (67.9%)	0.22	0.78
Alcoholics (n=52)	3 (5.8%)	21 (40.4%)	28 (53.8%)	0.26	0.74
Nondrinkers (n=64)	6 (9.4%)	18 (28.1%)	40 (62.5%)	0.23	0.77

ADH2, alcohol dehydrogenase 2

Table 5. Genotype and allele frequencies of *ADH3*

Group	Genotype			Allele frequency	
	ADH3*1/*1	ADH3*1/*2	ADH3*2/*2	ADH3*1	ADH3*2
Alcoholic cirrhosis (n=56)	50 (89.3%)	5 (8.9%)	1 (1.8%)	0.94	0.06
Alcoholics (n=52)	50 (96.2%)	2 (3.8%)	0 (0%)	0.98	0.02
Nondrinkers (n=64)	57 (89.1%)	7 (10.9%)	0 (0%)	0.95	0.05

ADH3, alcohol dehydrogenase 3

Table 6. Genotype and allele frequencies of *CYP2E1* in subjects with ADH2*2/*2, ADH3*1/*1, and ALDH2*1/*1

Group	Genotype			Allele frequency	
	A (c1/c1)	B (c1/c2)	C (c2/c2)	c1	c2
Alcoholic cirrhosis (n=35)	21 (60.0%)	13 (37.1%)	1 (2.9%)	0.79	0.21
Alcoholics (n=26)	18 (69.2%)	7 (26.9%)	1 (3.9%)	0.83	0.17
Nondrinkers (n=21)	11 (52.4%)	10 (47.6%)	0 (0%)	0.76	0.24

ADH2, alcohol dehydrogenase 2; ADH3, alcohol dehydrogenase 3; ALDH2, aldehyde dehydrogenase 2; CYP2E1, cytochrome P-4502E1

Table 7. Genotype and allele frequencies of *ADH2* in subjects with ADH3*1/*1, CYP2E1 c1/c1, and ALDH2*1/*1

Group	Genotype			Allele frequency	
	ALDH2*1/*1	ALDH2*1/*2	ALDH2*2/*2	ALDH2*1	ALDH2*2
Alcoholic cirrhosis (n=28)	2 (7.1%)	5 (17.9%)	21 (75.0%)	0.16	0.84
Alcoholics (n=29)	1 (3.4%)	10 (34.5%)	18 (62.1%)	0.21	0.79
Nondrinkers (n=18)	1 (5.6%)	6 (33.3%)	11 (61.1%)	0.22	0.78

ADH2, alcohol dehydrogenase 2; ADH3, alcohol dehydrogenase 3; ALDH2, aldehyde dehydrogenase 2; CYP2E1, cytochrome P-4502E1

Genotype and allele frequencies of *ADH2* in subjects with ADH3*1/*1, CYP2E1 c1/c1, and ALDH2*1/*1

Table 7 shows the genotype and allele frequencies of *ADH2* in subjects with ADH3*1/*1, CYP2E1 c1/c1, and ALDH2 *1/*1. Still, there was no significant difference among groups.

DISCUSSION

In the present study, we confirmed previous observations that the ALDH2*2 gene protects against the development of alcoholism (8, 9). However, we could not prove the hypothesis that habitual drinkers with ALDH2 deficiency may be at higher risk of alcoholic liver disease (22). This may be due to the relatively small sample size in our study. However, considering the low prevalence of ALDH2 deficiency among alcoholics as a whole, it is unlikely that the genotypes of *ALDH2* are the main determinant of alcoholic liver disease.

The effect of the c2 allele on the development of alcoholic liver disease is quite controversial (15-17). We could not confirm the previous observation by Tsutsumi et al. (15) that persons with the c2 allele are prone to the development of advanced alcoholic liver disease. These findings are consistent with the report of Chao et al. (17) in the Chinese population. Moreover, we found that the genotype and allele frequencies of *CYP2E1* in subjects with ADH2*2/*2, ADH3 *1/*1, and ALDH2*1/*1, analyzed to exclude the possible influence of ethanol-metabolizing enzymes other than CYP 2E1, showed no significant difference among patients with alcoholic cirrhosis, alcoholics without evidence of liver disease, and healthy nondrinkers. The increased transcriptional activity of the *c2* gene in vitro (7) may not reflect the activity in vivo. CYP2E1 phenotyping can be accurately assessed using chlorzoxazone metabolism in vivo (23). In studies using chlorzoxazone metabolism as a CYP2E1 probe, subjects heterozygous for the c2 allele did not show any difference in CYP2E1 activity at the basal level (23, 24). Furthermore, Lucas et al. (25) reported that patients with the mutated genotype are less inducible than those homozygous for the c1 allele after ethanol induction. So far, there is no direct evidence that allelic forms of the *CYP2E1* gene are related to the metabolic phenotypes.

Roles of polymorphisms of the ADH enzymes in the development of alcoholism are also controversial (9, 13, 14). Although the $\beta_2\beta_2$ and $\gamma_1\gamma_1$ isozymes of ADH have 40- and 2.5-fold higher Vmax than do the $\beta_1\beta_1$ and $\gamma_2\gamma_2$ isozymes in vitro (26), respectively, their effects on ethanol metabolism in vivo are not well-defined. Actually, no differences in the rate of alcohol elimination have been reported between individuals with the ADH2*1 and ADH2*2 (27). In the present study, we could not confirm the previous observation by Chao et al. that ADH2*2 and ADH3*1 alleles protect against the development of alcoholism in a Chinese population (9). This discrepancy may not have resulted from the small sample size, because the sample size needed for 90% certainty of detecting a reduction in allele frequency of ADH2*2 from 0.73 (in controls) to 0.51 (in alcoholics as a whole) with $p<.05$, as observed in the study by Chao et al. (9), is about 100 alleles (50 persons) in each group (i.e. control group and alcoholics group). The discrepancy between the Korean and Chinese population may be attributed to the different cultural background for drinking. In a cross-cultural study on drinking behavior between Koreans and Chinese, it was reported that traditionally Korean people usually encourage each other to drink in quantity, whereas controlled drinking behavior is more common in Chinese (28). Therefore, many Korean people have to drink against their wills to maintain their social relationships if their adverse symptoms are not as severe as in persons with ALDH defi-

ciency. However, a recent study (29) that analyzed a large European sample (876 samples) also showed that the ADH2 *2 allele decreases the risk for alcoholism (0.038, nonalcoholics; 0.013, alcoholics). Therefore, we could not completely exclude the possibility that the ADH2*2 allele protects from the development of alcoholism because our sample size is relatively small compared with that of the European study.

Because results are quite conflicting whether polymorphisms of ethanol-metabolizing enzymes are related with the risk of alcohol-related cirrhosis, a search for alternative candidate genes has been made recently. Because endotoxemia has been suggested to play an important role in the development of alcoholic liver disease, genes regulating cytokine expression have been studied as alternative candidate genes. To date, polymorphisms in the tumor necrosis factor promoter and the interleukin-1 receptor antagonist gene have been reported to be associated with the development of alcoholic liver disease (30, 31). Further studies are needed to confirm these findings.

In conclusion, we confirmed the observation that the *ALDH2*2* gene protects against the development of alcoholism. However, the development of cirrhosis in male Korean alcoholic patients was not associated with polymorphisms of the ethanol-metabolizing enzymes (ADH2, ADH3, CYP2E1, and ALDH2). In particular, the lack of association between ADH polymorphisms and the development of alcoholism might be due to the unique drinking culture in Korea.

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